1958-Plat
Opening the Protein Translocon
Ana Nicoleta Bondar, Douglas J. Tobias, Stephen H. White.
University of California at Irvine, Irvine, CA, USA.
The protein translocon (SecY in prokaryotes/Sec61 in eukaryotes) is essential for the biosynthesis of many proteins in all organisms. Opening of the translocon is thought to involve displacement of the plug helix that helps seal the translocon in its closed state; incorporation of transmembrane helices into the lipid membrane apparently involves opening of the lateral gate formed by transmembrane (TM) helices TM2 and TM7.
Our investigation of the molecular dynamics (MD) of the M. janaaschii SecYEG translocon indicated that the closed state of the translocon is stabilized by an exquisite network of inter-helical hydrogen bonds; this network also ensures that perturbations are rapidly relayed throughout the translocon, and cause displacement of the plug (1). Bioinformatics analyses and MD computations suggest an important functional role of the central cluster of hydrogen bonds that interconnect the gate helices and TM3.
Targeting of secreted proteins to the translocon is generally encoded in the signal peptide, a ~20 amino acids extension of the N-terminus of the nascent protein. MD simulations of translocons with bound proOmpA signal peptide reveal that inside the translocon the signal peptide interacts both with protein amino acid residues and water molecules. Hydrogen bonding between conserved SecY amino acids and the backbone of the signal peptide stabilizes the location of the signal peptide inside the translocon, and may explain the observation from our computations that mutating the translocon affects the structure and dynamics of the signal peptide.
Research supported in part by the National Institute of General Medical Sciences (GM-74637 and GM-86685 to S.H.W) and the National Science Foundation.
(CHE-0750175 to D.J.T.), and an allocation of computer time from the National Science Foundation through the TeraGrid resources at TACC (Ranger).
(1) Bondar A.-N., del Val C., Freites J.A., Tobias D.J., White S.H. Structure 18: 847-857, 2010.

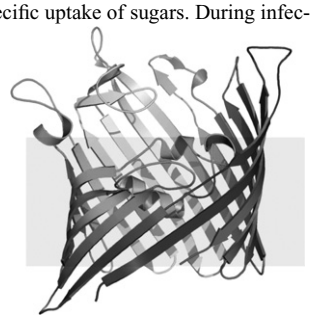
Symposium 16: 25 Years of Membrane Protein Structure

1959-Symp
Structures and Mechanisms of the Heme-Copper Containing Terminal Oxidases of the Respiratory Chain
Hartmut Michel.
Max Planck Inst Biophysic, Frankfurt, Germany.

1960-Symp
Architecture, Symmetry and Mechanism of Ionotropic Glutamate Receptors
Eric Gouaux[1,2].
[1]Oregon Health Sci Univ, Vollum Inst, Portland, OR, USA, [2]Howard Hughes Medical Institute, Chevy Chase, MD, USA.
Ionotropic glutamate receptors - AMPA, kainate and NMDA - are localized to the postsynaptic density and they mediate the majority of excitatory neurotransmission in the central nervous system by opening a transmembrane ion channel upon binding of glutamate. Despite their central role in signal transduction at chemical synapses, the principles of receptor mechanism, grounded on atomic structure of an intact receptor, are not well understood. Early work on the genetically excised agonist binding domain provided a simple model for glutamate-induced activation: agonist binding promotes closure of the clamshell-shaped binding domain. Further studies suggested that these binding domains are assembled as a dimer-of-dimers, a symmetrical arrangement different from the putative 4-fold, tetrameric symmetry of the ion channel domain. How are these different internal symmetries reconciled? To answer this question and to gain insight into the mechanisms of receptor function, we solved the atomic structure of the AMPA-sensitive rat GluA2 receptor complex. The receptor harbors an overall axis of two-fold symmetry and, as foreshadowed by studies on the isolated domains, the extracellular domains are organized as pairs of local dimers, with the ion channel exhibiting four-fold symmetry. The 2-fold (dimeric) and 4-fold (tetrameric) symmetry mismatch between the extracellular and ion channel domains, respectively, is mediated by two pairs of conformationally distinct subunits, A/C and B/D. Remarkably, the manner in which the A/C subunits are coupled to the ion channel gate is different from that of the B/D subunits, with the A/C subunits adopting a conformation distinct from the B/D subunits. The structural studies on glutamate receptors, together with vast knowledge of receptor function and biophysics, now allow us to develop mechanisms of function for AMPA, kainate and NMDA receptors based on three-dimensional, atomic resolution structure.

1961-Symp
Mechanism of G-Protein Coupled Receptor Activation
William Weis.
Stanford University School of Medicine, Stanford, CA, USA.
G protein coupled receptors (GPCRs) are seven-helix transmembane proteins that constitute the largest family of membrane proteins in the human genome. GPCRs are responsible for the majority of signal transduction events involving hormones and neurotransmitters across the cell membrane. Activating ligands (agonists) promote binding to and nucleotide exchange by a heterotrimeric G-protein, which dissociates and interacts with downstream effectors. Recent crystal structures have revealed inactive states of several GPCRs, but efforts to obtain an agonist-bound active-state GPCR structure have proven difficult due to the inherent instability of this state in the absence of a G protein. We generated a camelid antibody fragment (nanobody) to the human beta2-adrenergic receptor that exhibits G protein-like behavior, and obtained an agonist-bound, active-state crystal structure of the receptor-nanobody complex at 3.5 Angstrom resolution. Comparison with the inactive beta2-adrenergic receptor structure reveals subtle changes in the binding pocket, but these small changes are associated with an 11 Angstrom outward movement of the cytoplasmic end of transmembrane segment 6, and rearrangements of transmembrane segments 5 and 7 that are remarkably similar to those observed in opsin, an active form of rhodopsin. This structure provides insights into the process of agonist binding and activation of GPCRs.

1962-Symp
The Outer Membrane Protein PorB from Neisseria Meningitidis: from Structure to Function to Disease
Tina Iverson.
Vanderbilt, Nashville, TN, USA.
PorB is the second most prevalent outer membrane protein in Neisseria meningitidis, is required for neisserial pathogenesis and can elicit a Toll-like receptor mediated host immune response. The x-ray crystal structure of PorB has been determined to 2.3 Å resolution. Structural analysis and co-crystallization studies identify three putative solute translocation pathways through the channel pore: one pathway transports anions non-selectively, one transports cations non-selectively, and one facilitates the specific uptake of sugars. During infection, PorB likely binds host mitochondrial ATP, and co-crystallization with the ATP analog AMP-PNP suggests that binding of nucleotides regulates these translocation pathways both by partial occlusion of the pore and by restricting the motion of a putative voltage gating loop. PorB is located on the surface of N. meningitidis and can be recognized by receptors of the host innate immune system. Features of PorB suggest that Toll-like receptor mediated recognition outer membrane proteins may be initiated with a non-specific electrostatic attraction.



Symposium 17: CFTR: Structure and Function of an Anion Channel and the Molecular basis of Cystic Fibrosis

1963-Symp
Invited Speaker CFTR: Molecular Models of the Anion Conduction Path
David C. Dawson[1], Mark Sansom[2].
[1]OHSU, Portland, OR, USA, [2]Oxford University, Oxford, United Kingdom.
The cystic fibrosis transmembrane conductance regulator (CFTR) is an anion-selective channel that is expressed in human lungs, intestines, pancreas, liver, reproductive organs and a variety of glandular tissues. Chloride movement through CFTR is a key component of mechanisms for salt and water secretion in these organs. Mutations in the gene encoding CFTR cause cystic fibrosis, but the wild type channel is also a key element in secretory diarrheas such as cholera. CFTR belongs to the large, ABC transporter family, but it is, so far, the only member known to function as an ion channel.